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CARBOHYDRATE METABOLISM IN GREEN SWEET CORN DURING STORAGE AT DIFFERENT TEMPERATURES

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THE PROBLEM

The present paper deals with the character and kinetics of the processes involved in the rapid depletion of sugar in green sweet corn after it is separated from the stalk and more particularly with the relative rates of these processes at different storage temperatures, accurately controlled.

WORK OF PREVIOUS INVESTIGATORS

In the course of an extensive sweet corn investigation, Straughn [2] clearly shows that the loss of total sugars from green sweet corn is very rapid during the first 24 hours of storage at ordinary summer temperatures. Working with Stowell's Evergreen, he claims that about one-third of the total sugars disappeared during the first 24 hours of storage at a room temperature of about 25° C. A further loss occurred during the next 24 hours, but when the sugars reached 1.80 per cent no further loss was noted. This rate of sugar loss for the first 24 hours of storage at one uncontrolled temperature must be considered as merely an approximation, since analyses of different ears before and after storage were compared. The percentage of sugar in the different ears at the time of picking showed considerable variation. In the same paper it is concluded that there is no material advantage to be gained by storing the corn in a refrigerator. It should be noted, however, that the refrigerator showed a temperature of 23.5° C. during the first 24 hours and thereafter 17° C.

In a later paper by Straughn and Church [3] results are reported showing the change in the sugar content of green corn after a period of 36 hours' storage at room temperature. The data furnish very little additional information on this problem, as the experimental corn was secured upon the open market and the sugar loss in this corn had nearly ceased before

[1] The curves in figures 1 and 2 were drawn by John Paul Jones, of this laboratory.

[2] STRAUGHN, M. N. SWEET CORN INVESTIGATIONS. Md. Agr. Exp. Sta. Bul. 120, p. 37–78. 1907.

[3] STRAUGHN, M. N., and CHURCH, C. G. THE INFLUENCE OF ENVIRONMENT ON THE COMPOSITION OF SWEET CORN, 1905–1908. U. S. Dept. of Agr. Bur. Chem. Bul. 127, 69 p, 11 fig. 1909.

the experiment was begun. However, the data are interesting; they show the usual low sugar content of green corn as it is now purchased on the market. The percentage of sugar in this corn ranged from 1.70 to 1.49.

EXPERIMENTAL METHODS

One of the first problems to solve was a method by which the rate of the carbohydrate changes at different temperatures could be determined without comparing analyses of different ears. The following method was finally adopted: The ears for each experiment were brought to the laboratory within 15 minutes after picking and numbered consecutively. The first set of samples was taken from ears 1 and 2, and all ears were then placed immediately under the experimental conditions. At the end of 24 hours the second set of samples was taken from ears 1 and 2 and the first set from ears 3 and 4. After 48 hours the second set of samples was taken from ears 3 and 4 and the first set from ears 5 and 6. This overlapping method of sampling was continued every 24 hours until the experiment was completed. The change in chemical composition during each consecutive 24-hour period of storage could then be determined by comparing the analytical results of the first and second sets of samples from the same ear.

Stowell's Evergreen corn was stored at seven different temperatures—namely, 0°, 5°, 10°, 15°, 20°, 30°, and 40°C. All the temperatures were controlled within about 1°. The 30° temperature was controlled within 0.1°. The corn was stored with the husks on, and, in the case of the higher temperatures, the ears were placed in large desiccators, with the tubulure on the side left open to allow ventilation. Preliminary experiments showed that, as far as the carbohydrate changes are concerned, active aeration of the small number of ears used in each experiment was not important during the short experimental period of four days.

Under the conditions of the experiments there was very little change in the percentage of water in the corn at any temperature. However, the analytical results from the second set of samples were all calculated to the moisture of the first set in order to avoid false percentages due to loss or gain in water content during storage. In a few cases at the higher temperatures the percentage of water slightly increased on account of the accumulation of respiratory water and possibly water set free by condensation of polysaccharides.

ANALYTICAL METHODS

SAMPLING

Three rows of kernels were removed for each set of samples, care being taken to remove the entire kernel. In order to take the first set of samples, the husks were split lengthwise with a sharp knife and then cut

half way around at the base. After the kernels were removed the husks were brought back to place and held by means of rubber bands. For the second set of samples the husks were removed and three rows of kernels taken from the opposite side of the ear.

The corn was thoroughly ground to a mash in a small unglazed mortar and sampled immediately. On account of the short time required to sample the mash it was found unnecessary to surround the mortar with cracked ice. Each set of samples furnished material for the following determinations: Moisture, total sugars as invert sugar, sucrose, free-reducing substances, and starch. The starch was determined as glucose after hydrolysis with dilute acid.

MOISTURE.—Approximately 5 gm. of the mash were placed between tared watch glasses ground tight and held together by means of a clamp. After weighing, the cover glass was removed and the material covered with 1 cc. of alcohol. The samples were then dried to constant weight in a vacuum at 80° C. During the first drying a stream of warm, dry air was passed through the chamber. The watch glasses were clamped together before each weighing.

SUGARS.—When all things are considered, the alcohol method for the extraction of sugars from plant material in general seems preferable to any other yet devised. Since the procedure by different authors varies considerably, a large number of preliminary experiments were performed to determine the best procedure for the alcoholic extraction of sugars from the particular material at hand—namely, green sweet corn at different stages of maturity. The chief problem was to obtain complete extraction of the sugars and at the same time prevent any inversion of cane sugar as well as diastase action.

These experiments show that there is no appreciable hydrolysis of either sucrose or starch during boiling in 40 or 50 per cent neutral alcohol as long as 60 minutes. However, complete extraction was obtained by a much shorter period of boiling, and consequently the loss of alcohol during extraction is very much reduced.

The procedure finally adopted was as follows: Samples of 16 gm. each were weighed out into counterpoised 200 cc. Kohlrausch sugar flasks. A small amount of calcium carbonate was added to neutralize any acids liberated in the mash. It was later found that in the case of sweet corn this is not as important as in the case of many other plant tissues. The samples were covered immediately with 75 cc. of hot 95 per cent alcohol, the alcohol being previously measured into small boiling flasks and brought to boil on an electric hot plate. After the mixture began to boil on the steam bath, 50 cc. of hot water were added. This brought the extraction alcohol down to about 50 per cent. The water in the sample was taken into consideration in making this calculation. The foregoing method precluded any possible enzyme action in the weak alcohol while heating up to the boiling point. Small funnels were placed

in the necks of the flasks to condense the alcohol and the mixture was allowed to boil 30 minutes. While still hot, the flasks were made up to the mark with 95 per cent alcohol and allowed to stand over night. They were then shaken, again made up to the mark, tightly stoppered, and stored. The final strength of the alcohol in which the samples were stored was about 64 per cent.

When a large number of samples are taken during a comparatively short time, as was the case in this work, it becomes necessary to store most of the samples for some time. Since the storage problem is an important one, a number of experiments were conducted to determine the best treatment of the samples to prevent any carbohydrate changes during long periods of storage. The final method, previously described, was found to preserve the samples for at least 145 days without any appreciable carbohydrate changes. After boiling, the samples may be safely stored in 50 per cent alcohol. Cold treatment of the samples with 52 per cent alcohol inhibited invertase action, but there was considerable starch hydrolysis after a long period of storage. If the number of volumetric flasks is limited, a measured quantity of the filtered extract can be stored. In this work 150 cc. were frequently stored.

The method employed for the determinations of the sugars in the solutions was essentially the same as the one described by Bryan, Given, and Straughn.[1]

STARCH.—Ten gm. of the mash were weighed into counterpoised 200 cc. Erlenmeyer flasks and immediately covered with 50 cc. of 95 per cent alcohol. About 0.05 gm. of calcium carbonate was added and after thorough shaking the flasks were tightly stoppered and stored. The strength of the cold alcohol in the mixture was approximately 80 per cent. This was found sufficient to preserve the samples for several weeks without any appreciable change in the carbohydrates present. The method of weighing out the samples in small flasks, counterpoised on torsion balances sensitive to one-fifteenth gm. was found to give just as good duplicates as weighing the samples to the third place in weighing bottles. By the former method, the samples could be placed in alcohol in a very much shorter time.

The determinations were made according to the following procedure: Decant the preserving alcohol on to a 9 cm. No. 1 Whatman filter paper; add 75 cc. of 50 per cent alcohol and extract 24 hours at room temperature, shaking noon and evening; decant completely the 50 per cent alcohol; add 50 cc. more of the 50 per cent alcohol and allow to stand two hours, shaking three times; decant the alcohol and when all has run through the filter transfer the mash to the filter; apply suction and drain; add 50 cc. of 50 per cent alcohol to the flask to wash down

[1] BRYAN, A. Hugh, GIVEN, A., and STRAUGHN, M. N. EXTRACTION OF GRAINS AND CATTLE FOODS FOR THE DETERMINATIONS OF SUGARS; A COMPARISON OF THE ALCOHOL AND THE SODIUM CARBONATE DIGESTIONS. U. S. Dept. Agr. Bur. Chem. Circ. 71, 14 p. 1911.

the sides and transfer to the filter. With small portions of 50 per cent alcohol, transfer to the filter any material still remaining in the flask; after the alcohol has drained out of the filter, fill up once more with 50 per cent alcohol and drain. All sugars and any other reducing materials are now removed from the residue on the filter. The filter is filled twice with 95 per cent alcohol and the residue allowed to dry on the filter. The filter paper may be folded over the sample and placed in small stoppered vials for another period of storage if necessary.

The filter paper containing the sample was placed in a Kjeldahl flask and covered with 200 cc. of water; sufficient hydrochloric acid was added to give a final strength of acid in the mixture of 2.5 per cent. Hydrolysis was effected by boiling under a reflux condenser for three hours.

A number of the filter papers used for the filtration were hydrolyzed in the same strength of acid and for the same length of time as the samples. Although the papers were claimed by the manufacturers to be starch free, they were found to give a small amount of reducing material after hydrolysis. However, the amount of this material was consistent in all the boxes and in different parts of the box, so it was very easy to make the necessary correction for the filter paper in the final results. The starch was determined as glucose, but of course it includes any other polysaccharides which furnished reducing substances during the acid hydrolysis.

EXPERIMENTAL DATA

The work had not progressed far until it was evident that if the moisture in the corn at the time of picking had fallen below a certain percentage it became a factor in controlling the rate of sugar loss. In order to eliminate this variable factor, so that attention could be focused upon the temperature relation, the experimental ears were carefully selected to represent a fairly definite stage of maturity—namely, the typical milk or best eatable stage. Ears falling below 80 per cent water were excluded from the final calculations.

The work of the first year was repeated on another crop the succeeding year. The results of the two years' work are averaged in Table I. In the experiments of the first year, the carbohydrate changes for each consecutive 24-hour period were not determined in duplicate ears as described for the experiments of the second year. Each percentage in the table, therefore, is the mean of three ears, except in a very few cases where the results of one ear were excluded on account of the moisture content's falling below the arbitrary standard. The results of the experiments at 5° and 15° C. are not given, as they add nothing to the general conclusions. The average percentage of sugars in the corn at the beginning and end of each storage period is indicated by (*a*) and (*b*), respectively.

TABLE I.—*Loss of sugar from green sweet corn during consecutive 24-hour periods of storage at different temperatures*

ALL SUGARS

Number of hours in storage.	Ear lot.	Storage temperatures.									
		0° C.		10° C.		20° C.		30° C.		40° C.	
		Total.[1]	Loss.[1]	Total.[1]	Loss.[1]	Total.[1]	Loss.[1]	Total.[1]	Loss.[1]	Total.[1]	Loss.[1]
0	1*a*	5.91		5.83		6.17		5.34		6.72	
24	1*b*	5.43	0.48	4.83	1.00	4.59	1.58	2.65	2.69	3.64	3.08
24	2*a*	6.70		3.95		3.68		3.11		2.30	
48	2*b*	5.96	.74	3.43	.52	2.69	.99	2.68	.43	1.69	.61
48	3*a*	6.63		4.61		3.07		2.10		2.00	
72	3*b*	6.36	.27	3.92	.69	2.52	.55	2.03	.07	1.90	.10
72	4*a*	6.10		3.54		2.24		1.59			
96	4*b*	5.75	.35	2.93	.61	1.97	.27	1.49	.10		
		SUCROSE									
0	1*a*	3.87		3.77		3.68		3.68		4.50	
24	1*b*	3.73	0.14	3.00	0.77	2.54	1.14	1.50	2.18	2.18	2.32
24	2*a*	4.06		2.53		1.84		1.52		1.18	
48	2*b*	3.77	.29	1.99	.54	1.17	.67	1.24	.28	.76	.32
48	3*a*	4.49		2.74		1.38		1.02		1.05	
72	3*b*	4.25	.24	2.30	.44	1.12	.26	.97	.05	.91	.14
72	4*a*	3.84		1.87		1.11		.71			
96	4*b*	3.56	.28	1.41	.46	.85	.26	.67	.04		
		FREE-REDUCING SUBSTANCES									
0	1*a*	1.84		1.85		2.07		1.65		1.98	
24	1*b*	1.70	0.14	1.68	0.17	1.77	0.30	1.16	0.49	1.32	0.66
24	2*a*	1.66		1.29		1.68		1.56		1.06	
48	2*b*	1.55	.11	1.28	.01	1.41	.27	1.42	.14	.80	.26
48	3*a*	1.91		1.73		1.55		1.07		.90	
72	3*b*	1.89	.02	1.50	.23	1.28	.27	1.04	.03	.93	.00
72	4*a*	2.05		1.57		1.13		.88			
96	4*b*	2.00	.05	1.45	.12	1.04	.09	.81	.07		

[1] Total quantities of all sugars before and after storage and losses during storage are expressed in percentages.

The data in Table I show that the loss of sugar from corn during storage is not uniform, but becomes slower and slower as a final equilibrium is approached. The relative rates of processes of this kind at different temperatures can be determined accurately only by comparing

the times required to perform equal amounts of work at all temperatures, and not by comparing the amounts of work performed in equal times. In other words, we must compare the times required at the different temperatures to bring the process to the same stage. We are thus comparing stages where the ratio between the reacting material and the products is the same. Osterhout [1] has recently emphasized this point in a "Note on measuring the relative rates of life processes."

In order to make it possible to determine, on this basis, the relative rates of sugar loss at the different temperatures, the experimental results in Table I can be easily interpolated by a simple graphic method, to be described later, if they can be expressed in curves all starting from the same point.

This could be decided only atter a careful consideration of all the factors involved. If mass action alone were responsible for the gradual decline in the rate of sugar loss, then, at a given temperature, the average rate of change in any unit of time would be proportional to the sugar concentration. For a range of original sugar from about 4.5 to 7 per cent and of water from 78 to 80 per cent this was found to be the case for the first 48 hours of storage even at 30° C. (Table II).

TABLE II.—*Proportion of sugar lost during first 48 hours of storage at 30° C.*

Ear No.	Reducing sugar before storage.	Loss during storage.		Total sugar before storage.	Loss during storage.		Sucrose before storage.	Loss during storage.	
		Actual.	Proportional.		Actual.	Proportional.		Actual.	Proportional.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	1.70	0.68	37	6.16	3.59	58	4.24	2.77	65
2	2.40	1.02	42	7.20	3.87	54	4.80	2.95	61
3	2.60	1.05	40	6.74	3.88	58	4.14	2.89	69
4	.96	.22	43	4.47	2.59	58	3.33	2.33	69
5	1.66	.82	49	5.91	3.41	58	4.25	2.68	63
6	3.00	1.31	44	6.55	3.69	57	3.55	2.43	68

The sugar loss ceases when an appreciable amount of sugar is still present. Therefore, the speed of the counter process, that is, the formation of sugar, becomes a factor to be reckoned with when the processes have nearly reached an equilibrium. If at the beginning of storage the percentage of sugar in ear 1 is considerably greater than in ear 2, the latter would reach the equilibrium position sooner than ear 1. At the end of 72 hours of storage at 30° C. ear 1 might still have 2 per cent sugar while in ear 2 the sugar content might be only 1 per cent. The sugar loss in ear 2 being nearer the equilibrium point, the speed of the counter process would be greater in this ear than in ear 1. Therefore, during the next 24 hours the proportionality between the sugar lost and the sugar

[1] OSTERHOUT, W. J. V. NOTE ON MEASURING THE RELATIVE RATES OF LIFE PROCESSES. *In* Science, n. s., v. 48, no. 1233, p. 173–174, 3 fig. 1918.

present would not be the same in the two ears. This was proved experimentally.

In considering the rate of the counter reaction in connection with the problem at hand—namely, the possibility of expressing the experimental results in curves all starting from the same point—it must be borne in mind that it becomes appreciable only near the point of equilibrium, and even then it would affect the proportionality between the sugar present and the sugar lost in different ears at the same temperature only when the percentage of sugar in the ears at the beginning of storage varied considerably.

A decrease in the quantity of active enzymes present would produce a steady fall in the values of the velocity constants; this would cause a decreasing rate of actual sugar loss. There is no evidence that this occurs up to 30° C.

In view of the foregoing facts, together with the fact that the ears selected for the final calculations were all in practically the same stage of maturity and therefore contained nearly the same percentage of original sugar, the following procedure in preparing the data for construction of curves all starting from the same point seemed justified. The sugar lost during each 24-hour period of storage was calculated as proportions of the sugar present in the ears at the beginning of each period. The percentages of sugar found in the ears analyzed at the beginning of each experiment were then averaged. Ten ears with not less than 80 per cent water were included in the final average.

Taking this mean as the starting point for all temperatures and applying the proportions of sugar lost during each succeeding 24-hour period, calculated from the experimental data, a new set of proportions was obtained, based upon the same original sugar content in all cases. A single concrete case may serve to clarify the foregoing procedure. The total sugar in all the ears analyzed at the beginning of each experiment averaged 5.766 per cent. During the first 24 hours of storage at 30° C. the average loss of total sugar in three ears was 50.28 per cent of the initial sugar present; that is, the total sugar in the corn was 50.28 per cent less than at the beginning of storage. Applying this proportion to an initial sugar content of 5.766 per cent, we obtain, after the first 24-hour period of storage at 30°, a total sugar content of 2.867 per cent. Making use of all the experimental proportions in the same manner, the percentage of total sugar present at the end of each 24-hour period of storage was calculated, assuming that the sugar content at the beginning of storage was 5.766 per cent. Each calculated percentage was then substracted from 5.766, the initial sugar present. The sugar loss, expressed as percentages of the initial sugar, could then be calculated for the following storage periods: 24, 48, 72, and 96 hours. The same procedure was followed for all the sugars at all the temperatures, with the results shown in Table III.

TABLE III.—*Sugar loss from green sweet corn during different periods of storage at different temperatures, expressed as percentages of the same initial sugar at all temperatures*

TOTAL SUGARS

Number of hours in storage.	Storage temperature.				
	0° C.	10° C.	20° C.	30° C.	40° C.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
24	8. 12	16. 98	25. 61	50. 28	45. 79
48	14. 51	27. 95	45. 73	57. 09	60. 15
72	18. 03	38. 71	55. 50	59. 00	62. 16
96	22. 00	49. 22	62. 10	61. 84	.....

SUCROSE

24	3. 51	20. 78	31. 05	59. 42	51. 03
48	10. 39	37. 49	56. 12	66. 76	64. 68
72	15. 08	47. 46	64. 22	68. 55	69. 24
96	21. 25	60. 54	70. 16	70. 19	.....

FREE-REDUCING SUBSTANCES AS INVERT SUGAR

24	7. 58	9. 26	14. 72	29. 96	33. 48
48	13. 61	10. 52	28. 84	36. 19	49. 76
72	14. 62	16. 71	40. 79	39. 74	49. 76
96	16. 97	23. 33	45. 65	43. 19	.....

The data in Table III, showing the rate of actual loss for total sugars and sucrose, were plotted as curves (fig. 1 and 2).

The curve for 0° C. shows a more rapid sugar loss than is typical for this temperature. In the first place, it required some time for the corn to cool down to this temperature. At the end of each 24-hour period a pair of ears were removed from the cold chamber in order to take the first set of samples. Although the sampling period was short, the temperature of the corn would soon rise a few degrees above 0°. The loss of sugar at the sampling temperature is accumulative in the curve.

The inversion of sucrose appears to be the controlling process in the sugar loss, as the curves for the decrease of sucrose are very similar to those for the loss of total sugar.

TEMPERATURE COEFFICIENT.—Since the curves in figures 1 and 2 all start from the same point, by means of a simple graphic method the relative rates of sugar loss at the different temperatures can now be determined by comparing the times at different temperatures required to do the same amount of work. As an illustration we will choose a stage in the depletion of sugar when 40 per cent of the total sugar is lost; in other words, at this point the sugar in the corn is 40 per cent less than at the beginning of storage. A horizontal line is drawn from

this point through all of the curves. Vertical lines are now dropped from the points of intersection to the base line. The times in hours required at the different temperatures to bring the sugar loss to this point are read off on the base line (see fig. 1). The procedure was repeated for all the percentages given on the ordinate.

The relative rates of sugar loss at the different temperatures are expressed in Table IV as the reciprocals of the times in hours required to bring the process to five different stages. The temperature coefficients were obtained from these reciprocals. The results at 40° C. were not

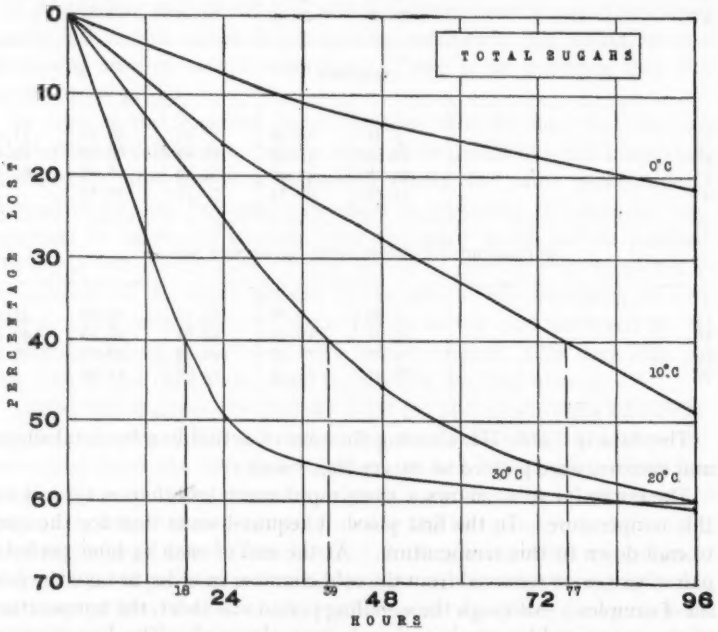

FIG. 1.—Depletion of total sugars in green sweet corn during consecutive 24-hour periods of storage at different temperatures. The ordinates are given by the numbers on the left of the figure and represent the loss of sugar expressed as percentages of the initial sugar, which was 5.91 per cent, wet weight.

included in the foregoing calculation as there was evidently destruction of the enzymes or other alteration in the system by the high temperature.

Some of the curves for the sugar loss, expecially those for sucrose, approach true logarithmic curves; and satisfactory constants were obtained for most of the storage period by applying the simple uni-molecular equation. During the latter part of the period there was a falling off in the velocity constants, due no doubt to the counter reaction. The simple uni-molecular equation assumes that the reaction proceeds to completion or so near completion that the speed of the counter may be ignored. However, as Osterhout has shown in the paper previously

cited, it is not necessary to determine the true velocity constants of a process under different conditions if only the relative rates are desired. This may be accomplished in the manner indicated in Table IV by comparing the reciprocals of the times required to do the same amount of work.

In general, it may be stated that up to 30° C. the rate of sugar loss in green corn is doubled for every increase of 10°. This applies to both total sugars and sucrose. It should be noted, however, that between 0° and 10° the temperature coefficient for sucrose is considerably greater than 2.

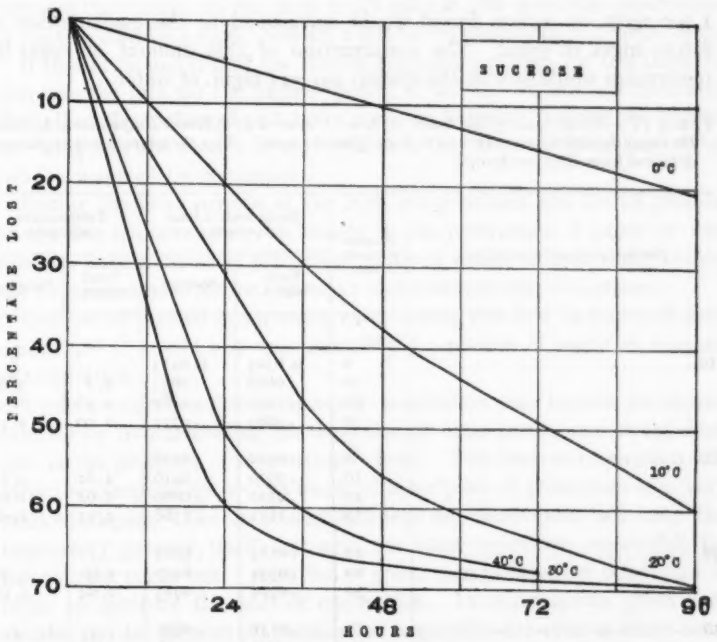

FIG. 2.—Depletion of sucrose in green sweet corn during consecutive 24-hour periods of storage, expressed as percentages of the initial sucrose in the corn, which was 3.87 per cent, wet weight.

RESPIRATION.—In a former paper the writer [1] has shown that respiration in green sweet corn after it is first pulled from the stalk is comparatively high. During the first 24 hours of storage at 30° C. the corn with the husks removed respired at an average rate of 50 mgm. of carbon dioxid per kgm. per hour. This rate became slower and slower until it reached, in eight days, a constant rate of about 18 mgm. of carbon dioxid per kgm. per hour. Respiration of course consumes sugar and therefore accounts for some of the depletion of sugar in sweet corn

[1] APPLEMAN, Charles O. RESPIRATION AND CATALASE ACTIVITY IN SWEET CORN. *In* Amer. Jour. Bot., v. 5, p. 207–209, 1918.

during storage. During each consecutive 24-hour period of storage the percentage of sugar in the corn, however, is only slightly altered by respiration, as shown by the following illustration. Straughn, in the paper previously cited, averaged the weight of kernels and cobs from 18 ears and found that the kernels averaged approximately 50 per cent of the total weight. If we assume that all of the carbon dioxid came from the kernels, then 500 gm. of kernels would produce 1,200 mgm. of carbon dioxid during the first 24 hours' storage at 30°. From the formula

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

1,200 mgm. of carbon dioxid would correspond to the consumption of 818.61 mgm. of sugar. The consumption of this amount of sugar by respiration would free in the system 491.343 mgm. of water.

TABLE IV.—*Reciprocals of the times, in hours, required at different temperatures to bring the sugar depletion in sweet corn to five different stages. Also the temperature coefficients obtained from these reciprocals*

Percentage of initial sugar lost.	Storage temperature.	Reciprocals of time periods.		Temperature coefficients.	
		Total sugars.	Sucrose.	Total sugars.	Sucrose.
	°C.				
10	0	0.0303	0.0213	.....	.....
	10	.0666	.0833	2.2	3.91
	20	.1041	.1282	1.56	1.53
	30	.2083	.2777	2.00	2.16
20	0	.0122	.0108	.....	.....
	10	.0320	.0416	2.62	3.85
	20	.0520	.0666	1.62	1.60
	30	.1111	.1388	2.13	2.08
30	10	.0184	.0268	.....	.....
	20	.0354	.0427	1.92	1.59
	30	.0724	.0925	2.04	2.16
40	10	.0131	.0191	.....	.....
	20	.0252	.0326	1.92	1.71
	30	.0555	.0694	2.30	2.12
50	10	.0101	.0136	.....	.....
	20	.0173	.0256	1.71	1.88
	30	.0406	.0555	2.34	2.17
60	10	.....	.0104	.....	.....
	20	.....	.0171	.....	1.64
	30	.....	.0416	.....	2.43
Average temperature coefficient		.....	.....	2.04	2.14

For the sake of simplicity, we will confine the system to 100 gm. of corn and suppose that at the beginning of storage it contained 5 gm. or 5 per cent sugar and 80 gm. or 80 per cent water, a fair average for the

corn used in this work. According to the foregoing rate of respiration this system would lose 163.72 mgm. of sugar during the first period of storage of 24 hours. At the same time 98.269 mgm. of water would be freed in the system. Our system would now contain 80.0983 gm. of water and 4.8363 gm. of sugar. By correcting for the slight loss of dry matter, the system would contain 80.1507 per cent water and 4.8395 per cent sugar. These percentages would be those found by actual analysis of the 100 gm. of corn after 24 hours' storage, assuming that no other changes occurred besides respiration.

If we now calculate the percentage of sugar on the basis of the original water in the system, as was done in all cases in this work, the percentage of sugar would be 4.8726, showing a loss by respiration of 0.1274 per cent.

It should be noted that the rate of respiration chosen for this illustration was the rate for the highest period at 30° C. It was also assumed that all of the carbon dioxid came from the kernels. During the immature stages of the corn it is very probable that some of the sugar in the cob is consumed by respiration.

During the later periods at the high temperatures and for all periods at the low temperatures, the change in the percentage of sugar by respiration during the short periods of 24 hours would be practically within the experimental error for the sugar and moisture determinations.

One ton of husked green sweet corn, during the first 24 hours of storage at 30° C. would lose approximately 3.2 pounds of sugar on account of respiration.

Under certain conditions, however, respiration may become an important factor in accelerating the depletion of sugar from green sweet corn. One of the products of respiration is heat. This heat of respiration will raise the temperature on the inside of large piles of green corn to a very marked degree. The increased temperature accelerates not only the respiratory process itself but also the other processes responsible for most of the sugar loss. Aeration of green corn is therefore important in order to dissipate the heat of respiration. In other words, green corn should not be allowed to remain in large piles for even a short time, especially during midsummer temperature.

STARCH FORMATION.—If the sugar is all converted into starch or other polysaccharides, hydrolyzed by dilute acids, then the sum of the total sugars and the polysaccharides as glucose should be the same before and after storage. During the first period there is a slight deficit after storage, especially in the more immature ears. A part of this deficit is due to the high respiration of this period; but some of it is probably accounted for, in the immature ears when the sugar is high, by the formation of cellulose. During the later periods of storage many of the ears, depending largely upon the stage of maturity, show a slight increase in the sum of the total sugars and polysaccharides. This is true especially at the higher temperatures and is probably accounted for by the

sugar of the cob being drawn into the grain for starch formation as the sugar in the grain is depleted. Analyses of cobs from immature ears gave a total sugar content of about 7 per cent. The sugar in the cob decreased slightly during storage, but there was no starch formation in the cob.

After noting these exceptions, which alter the balance only slightly, it may be stated in general that most of the sugar loss in green sweet corn is balanced by the gain in polysaccharides, chiefly starch (Table V).

TABLE V.—*Depletion of sugar in green sweet corn balanced chiefly by formation of polysaccharides hydrolyzed by dilute acid*

Ear No.	Total sugars plus polysaccharides as glucose.							
	First period.		Second period.		Third period.		Fourth period.	
	0 hours.	24 hours.	24 hours.	48 hours.	48 hours.	72 hours.	72 hours.	96 hours.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0° C.								
1	11.01	11.04	12.40	12.39	11.07	11.29	13.57	13.56
2	10.72	10.67	14.84	15.35	.....	.....	13.41	12.68
Average.	10.86	10.86	13.62	13.87	11.07	11.29	13.49	13.12
10° C.								
1	14.56	14.53	15.26	15.89	15.86	15.35	14.29	15.05
2	11.79	11.37	14.01	13.50	11.93	11.58	10.81	10.49
Average.	13.18	12.95	14.64	14.70	13.89	13.47	12.55	12.77
20° C.								
1	11.50	11.09	15.19	15.13	11.28	10.56	10.93	10.46
2	10.82	9.97	13.64	13.58	10.21	10.24	13.34	13.76
3	.....	.....	12.13	11.20	12.67	12.20	.....	.....
Average.	11.16	10.53	13.65	13.30	11.35	11.00	12.14	12.11
30° C.								
1	14.75	14.10	13.39	13.55	13.30	13.32	12.48	12.66
2	12.20	10.53	16.77	17.24	12.16	12.66	12.17	12.61
3	.....	.....	13.13	12.01	10.80	12.36	11.43	12.17
Average.	13.48	12.31	14.43	14.26	12.08	12.78	12.02	12.48

SUMMARY

The data recorded in this paper apply to Stowell's Evergreen corn, picked in the typical milk or best eatable stage and having a water content of approximately 80 per cent.

A method was devised by which the rate of sugar loss from green sweet corn could be determined for consecutive 24-hour periods of storage by comparing analyses of corn from the same ear.

The depletion of sugar in green sweet corn after it is separated from the stalk does not proceed at a uniform rate but becomes slower and slower until finally the loss of sugar ceases when the initial total sugar has decreased about 62 per cent and the sucrose about 70 per cent. Calculated on the basis of original moisture, the corn contained, when the depletion of sugar ceased, approximately 1.5 per cent total sugar as invert sugar, 0.7 per cent sucrose, and 0.8 per cent free-reducing substances. The actual percentage of sugars would depend upon the amount of water in the corn after storage. Under the experimental conditions there was very little change in the percentage of water in the corn employed in this work.

Reversibility of the chief processes involved in the sugar depletion, resulting in an equilibrium between the rate of sugar loss and the rate of sugar formation, would account for the cessation of actual sugar loss.

During the early periods of storage, the falling off in the rate of actual sugar loss is due to mass action. When the equilibrium is nearly reached the counter reaction, that is the formation of sugar, also tends to slow up the rate of sugar loss. Any destruction or decrease in the quantity of enzymes present would produce a falling off in the value of the velocity constant, with a consequent decrease in the rate of actual sugar loss. There is no evidence that this occurs up to 30° C. At 40° there is actual destruction of the enzymes or other alteration on the system. The rate of actual sugar loss must not be confused with the velocity constant.

Raising the temperature simply hastens the attainment of the equilibrium positions, which seem to be about the same for all temperatures. At 30° C., 50 per cent or most of the total sugar loss occurs during the first 24 hours of storage. At 20°, 25 per cent, and at 10°, or good refrigerator temperature, only about 15 per cent is depleted during the same period.

Relative rates at different temperatures, of processes that become slower and slower until an equilibrium is reached, can be accurately determined throughout this entire course only by comparing the times required to bring the process to the same stage at all temperatures. In order to make this comparison possible the experimental results were interpolated by a simple graphic method. The temperature coefficient was then obtained by comparing the reciprocals of the times

required to do the same amount of work at the different temperatures. In this manner the temperature coefficients were determined for six different stages. Up to 30° C. an average coefficient of 2.03 was obtained for the loss of total sugars and 2.14 for sucrose. From 0° to 10° it was greater than 2 in the case of sucrose.

In general, it may be stated that the rate of sugar loss, until it reaches 50 per cent of the initial total sugar and 60 per cent of the sucrose, is doubled for every increase of 10° up to 30° C.

Respiration in green corn is comparatively high when the corn is first picked but falls off rapidly with storage. This process, however, accounts for only a small part of the actual decrease in the percentage of sugar in the corn during the consecutive 24-hour periods of storage even at 30° C. One ton of husked green sweet corn during the first 24 hours of storage at 30° would lose approximately 3.2 pounds of sugar on account of respiration.

Respiration may become indirectly a more important factor in accelerating the depletion of sugar by raising the temperature on the inside of large piles of green corn.

Most of the decrease in the percentage of sugar in green sweet corn during storage is due to condensation of polysaccharides, chiefly starch.

CERTAIN RELATIONSHIPS BETWEEN THE FLOWERS AND FRUITS OF THE LEMON[1]

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The physiological characteristics of the cultivated lemon make it an interesting object for study, since its period of blossoming and fruiting extends through much of the year. On most lemon trees it is possible to find all stages of development between blossoms and mature fruit throughout the year, though in varying amounts. There are distinct cycles in both the vegetative and fruiting activities of this tree whose limits are recognized by those engaged in its cultivation. One of the objects of the present study was to obtain quantitative records of these cycles and especially of the relations between flowers and fruit.

The present study attempts to discuss:

(*a*) The seasonal distribution of the fruit buds;

(*b*) The size and productiveness of the inflorescences;

(*c*) The time required for the growth of fruit and the relation of this time to the season at which the buds appear;

(*d*) The numerical ratio of flower buds to mature fruit.

The material studied consisted of a small group of Lisbon lemon trees located on the Limoneira ranch near Santa Paula, Calif. The trees stand in the midst of a large lemon grove and have received good orchard treatment with respect to cultivation, irrigation, and so forth. No especial attention in these particulars was given to the trees during the time observations were being made. All were free from injurious insects and fungous diseases. Each month for one year approximately 50 fruit twigs bearing fruit buds ready to open were selected and marked with identification tags. The twigs were chosen on seven adjacent trees, six of which were full-bearing trees 22 years old. The seventh was 6 years old, but was very fruitful. As soon as a twig was selected an entry was made on a special blank on which full records could be subsequently kept concerning leaves, buds, fruits, and new twigs. Once a month the twigs were examined and the data recorded on special blanks.

When the first year ended 12 lots of twigs had been selected and marked, and from that time the records on all twigs were continued for another year. Thus the first twigs selected were under observation for two years and the last for one year. A total of 610 twigs was selected and observed, but there was some loss due to the removal of tags by winds, so that the final number was somewhat less than 600. The partial records of twigs

[1] Paper No. 54, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

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whose tags disappeared were discarded. The writer is indebted to the management of the Limoneira Co. for their friendly cooperation in this work, as well as to various members of the staff of the Citrus Experiment Station for their assistance in the tedious work of obtaining and compiling data.

All biological work, especially work done in the field, is accompanied by inevitable error. The present is no exception. In August, 1916, much of the small fruit on these trees was killed during their fumigation with hydrocyanic-acid gas to kill insects. The following November the trees blossomed profusely, perhaps due to the earlier loss of a portion of their crop. In the early months of 1917 some of the small fruit was killed by freezing temperatures, in spite of the fact that oil heaters were used in the grove and all vigilance was exercised to avoid losses. In June, 1917, following a period of very hot weather, much of the more mature fruit fell from the trees. It might be thought advisable to discard a portion of the records which are known to be subject to these errors, but why should one discard errors due to climatic conditions which he recognizes while retaining other possibly greater errors which he does not recognize?

SEASONAL DISTRIBUTION OF FRUIT BUDS

The lemon tree continually produces fruit buds, yet their distribution through the year is not uniform. Information upon their seasonal distribution was obtained from the data for 4,545 "new buds"; that is, buds which appeared on twigs subsequent to the selection of these twigs for the purpose of study. These data were used to avoid the results of conscious or unconscious selection by the person who chose and tagged the original twigs. For example, because large clusters of buds are more conspicuous, a larger percentage of the fruit buds on the tree may have been chosen at one time than at another. The effect of this would have been to give larger records at one season and lower at another. It should be remarked, however, that since we were dealing with what is recognized as "fruit wood" the average number of buds on the twigs selected might be higher than for the average twig of the tree.

The figures given in Table I show the percentage of the new buds which were produced in the different months of the year and are based upon the observations of 4,545 buds during a period of two years.

TABLE I.—*The distribution of lemon buds by months*

[Average for 1916 and 1917]

Month.	Buds (percentage of total).	Month.	Buds (percentage of total).
January	0.31	July	4.81
February	.37	August	2.08
March	29.74	September	1.18
April	36.13	October	1.83
May	4.55	November	13.11
June	3.00	December	2.56

A survey of this table and of figure 1 shows that there are two periods in the year at which fruit buds were principally produced. In round numbers, about 66 per cent of the buds appeared in March and April, about 13 per cent appeared in November, and 20 per cent between April and November. There was, therefore, a very pronounced seasonal distribution of fruit buds on the trees observed.

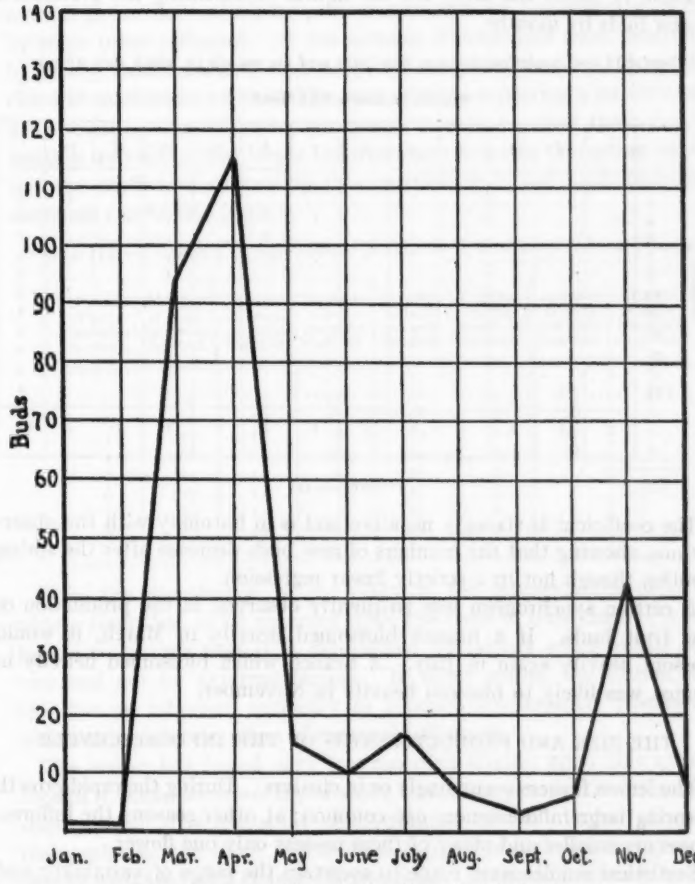

FIG. 1.—Average monthly production of lemon buds during the year.

The appearance of large numbers of fruit buds in March and April is undoubtedly related to the greater activity of the tree, following its slower winter growth. The secondary maximum in November, following the last growth cycle of the tree for the season, is not so easy of explanation. It might be assumed that the appearance of buds at this time was a reaction to the large supply of elaborated food material in the tree

and that the tree responded by putting forth fruit buds, while hindered by climatic conditions from producing vegetative growth.

In view of the fact that there are two periods of the year in which there is a maximum production of buds, it is logical to expect that the coefficient of correlation between time and number of buds would be negative, since the maximum production is in the early part of the year.

Table II shows the array for the figures representing the production of new buds by months.

TABLE II.—*Correlation between new buds and the month in which they appear*

Mean number of new buds per 50 twigs by months	Months, beginning with March												
	1	2	3	4	5	6	7	8	9	10	11	12	
1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	1	2
4	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	1
6	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	1
7	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	1
8	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	1
10	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	1
15	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
16	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	1
42	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	1
95	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
115	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
	1	1	1	1	1	1	1	1	1	1	1	1	12

$r = -0.650 \pm 0.112$

The coefficient is strongly negative and is in harmony with the observations, showing that the numbers of new buds decrease after the spring months, though not in a strictly linear regression.

A certain synchronism was frequently observed in the production of new fruit buds. If a branch blossomed heavily in March, it would blossom heavily again in July. A branch which blossomed heavily in August was likely to blossom heavily in November.

THE SIZE AND PRODUCTIVENESS OF THE INFLORESCENCES

The lemon flowers occur singly or in clusters. During the rapid growth of spring large inflorescences are common; at other seasons the inflorescences are smaller and many of them possess only one flower.

Statistical studies were made to ascertain the range of variability and the productiveness of the inflorescences on these particular lemon trees. Data on 1,363 inflorescences which appeared during the course of the observations were examined. The number of flowers per inflorescence ranged from 1 to 28. The relative frequency of the inflorescences in relation to the number of buds per inflorescence is shown in Table III. The data show that the greatest frequency occurred in the class of inflorescences which bore a single bud and that the frequencies decreased quite

uniformly as the number of buds per inflorescence increased in succeeding classes. From these data the following constants were calculated:

Mean number of buds per inflorescence = 4.784 ± 0.071.

Standard deviation = 3.916 ± 0.050.

Coefficient of variability = 81.86 ± 1.62.

An inspection of the figures shows several interesting relationships. The number of buds per inflorescence shows no tendency whatever to follow the normal curve of errors; therefore we may conclude that the number is not determined by pure chance, but, on the contrary, is fixed by some other influence. If the number of buds had been determined by purely casual factors, such as position on the tree, age of wood, or climatic conditions, we should be warranted in expecting a purely chance distribution. In a following paragraph it is shown that there is a correlation indicating that larger inflorescences occur in the spring months, but the coefficient expressing this correlation is not such that much emphasis can be laid upon it.

TABLE III.—*Frequency of inflorescences in relation to number of buds they produced*

Number of buds per inflorescence.	Number of inflorescences observed.	Number of buds per inflorescence.	Number of inflorescences observed.	Number of buds per inflorescence.	Number of inflorescences observed.	Number of buds per inflorescence.	Number of inflorescences observed.
1	239	9	51	17	7	25	2
2	216	10	37	18	4	26	1
3	173	11	18	19	2	27	1
4	168	12	20	20	0	28	2
5	134	13	18	21	0		
6	125	14	7	22	3		1,363
7	62	15	7	23	2		
8	53	16	11	24	0		

Since the distribution of the buds on the inflorescences departs so widely from that to be expected upon the basis of pure chance, it seems logical to assume that it is determined by factors which reside in the tree and not by external factors. In other words, the Lisbon lemon tree has an inherited tendency to produce few-flowered inflorescences which outweighs the effect of external influences.

The writer has found very few recorded studies upon this question, though it would seem worthy of study both from practical and theoretical standpoints. The frequency of distribution of the number of seeds in receptacles of the lotus (*Nelumbium luteum*) was found to agree very closely with that of a chance distribution.[1] It should be noted, however, that the two cases differ in the morphology of the organs in question. In lotus we are dealing with an organ developing from a compound ovary—that is, with one flower; but in the lemon inflorescence we are dealing with a short branch bearing flowers. It is possible that the

[1] PEARL, Raymond. VARIATION IN THE NUMBER OF SEEDS OF THE LOTUS. *In* Amer. Nat., v. 40, no. 479, p. 757–768, 4 fig., 1906.

number of seeds developed from a compound ovary is dependent upon a set of external, casual factors, such as amount or variability of pollen, conditions under which pollination occurs, and access of the mother plant to suitable supplies of nutriment. On the other hand, the number of flowers produced on an inflorescence may be more largely predetermined in the mother plant by such internal factors as those which determine the position and arrangement of leaves and others which act to produce generic and specific characters.

The study of the inflorescence may be carried a step further by attempting to determine whether the larger inflorescences were more characteristic of one season than of another. If so, it might show whether the size of the inflorescence is in any way influenced by seasonal conditions. Data for 403 inflorescences were available and represented a fair random sample as far as seasonable distribution is concerned. Table IV shows the correlation between the average number of new inflorescences on the seven trees and the average number of buds per inflorescence. It seemed more nearly correct to make this sort of correlation than one between months of the year and number of buds per inflorescence, since it eliminates irregular regression due to periodicity, leaving numbers of buds as the two factors for correlation.

TABLE IV.—*Correlation between monthly average size of inflorescence and numbers of inflorescences produced*

Average number of new inflorescences on seven trees per month	Average size of inflorescence (number of buds)												
	1.1	1.3	1.6	1.8	1.9	2.3	3.3	4.6	4.7	4.9	5.0	5.4	
2											1		1
4		1											1
6				1					1				2
9	1												1
12												1	1
18			1										1
33							1						1
40					1								1
44						1							1
59								1					1
170										1			1
	1	1	1	1	1	1	1	1	1	1	1	1	12

$r = 0.351 \pm 0.171$

The average number of new inflorescences per month on the seven trees ranged from 2 to 170; the average size by months ranged from 1.1 buds to 5.4 buds. The coefficient of correlation between these factors is 0.351 ± 0.171. Since the coefficient is only twice its probable error we must regard it as rather doubtful in indicating a correlation between these factors. It may be taken, however, to indicate that larger inflorescences were more abundant in seasons in which the number of new buds

was greatest—namely, in the spring months, or at other times at which the activity of the tree is at its height. This conclusion is in agreement with the repeated observation that thrifty trees most commonly bear lemons in clusters.

The next question to be investigated was one of considerable physiological interest: What is the correlation between the number of buds per inflorescence and the numbers of fruits matured per inflorescence?

A positive correlation approaching 1 is to be expected in case buds on all sizes of inflorescences have equal chances of development; a value much below this indicates that a bud on a larger inflorescence has a poorer chance. It is certain from the nature of the case that there must be some relationship between the two, since an inflorescence having only 1 flower could not produce more than 1 fruit, but an inflorescence possessing 20 flowers may or may not mature a proportional number of fruits. If we take the fruits whose history could be definitely ascertained and arrange them with regard to the size of the inflorescence from which they developed, we get the arrangement shown in Table V.

TABLE V.—*Correlation between numbers of buds and fruit on inflorescences of various sizes*

Buds per inflorescence	Fruits matured per inflorescence						
	0	1	2	3	4	5	
1	217	22	.....	.....	.....	.....	239
2	186	29	1	.....	.....	.....	216
3	144	27	2	.....	.....	.....	173
4	133	27	7	1	.....	.....	168
5	107	22	4	1	.....	.....	134
6	96	23	6	.....	.....	.....	125
7	50	4	5	1	1	1	62
8	44	7	1	1	.....	.....	53
9	38	8	5	.....	.....	.....	51
10	31	4	2	.....	.....	.....	37
11	12	5	1	.....	.....	.....	18
12	14	4	2	.....	.....	.....	20
13	14	3	1	.....	.....	.....	18
14	5	1	1	.....	.....	.....	7
15	5	.....	2	.....	.....	.....	7
16	5	2	4	.....	.....	.....	11
17	6	.....	.....	.....	1	.....	7
18	3	.....	1	.....	.....	.....	4
19	2	.....	.....	.....	.....	.....	2
22	3	.....	.....	.....	.....	.....	3
23	2	.....	.....	.....	.....	.....	2
25	2	.....	.....	.....	.....	.....	2
26	1	.....	.....	.....	.....	.....	1
27	1	.....	.....	.....	.....	.....	1
28	.....	.....	1	.....	1	.....	2
	1,121	188	46	4	3	1	1,363

$r = 0.178 \pm 0.017$

As determined from these figures, the coefficient of correlation is 0.178 ± 0.017, indicating a positive correlation between the size of the inflorescence and the number of fruits it brings to maturity. The small value of the coefficient, however, is worthy of note. It was previously mentioned that we should expect upon *a priori* grounds a definite relationship between flowers and fruits, especially in the case of the smaller inflorescences. It will therefore be in order to inquire whether the larger inflorescences are relatively as fruitful as the smaller.

In order to secure a measure of the relative fruitfulness of the inflorescences, I have used a formula which Harris [1] published several years ago. The correlation coefficient as here used is intended to measure the correlation between the number of fruits produced per inflorescence and the deviation of this number from its probable value, in case the number of fruits per inflorescence is in the same proportion to the number of buds per inflorescence as the total number of fruits to buds in the entire population. It is computed from the formula,

$$r_{zs} = \frac{r_{xy} - V_x/V_y}{\sqrt{1 - r^2_{xy} + (r_{xy} - V_x/V_y)}}$$

where x = buds and y = fruits per inflorescence, V_x and V_y are the coefficients of variability of the two characters, and z is to be read as "the deviation of the number of fruits per inflorescence from its probable value."

The value of this correlation as computed for the lemon inflorescence is $r_{zs} = 0.183 \pm 0.018$.

This negative value of r_{zs} is interpreted to mean that there is a distinct negative correlation between the size of the inflorescence and its power to develop its buds into fruits. In other words, a bud on one of the smaller inflorescences has a greater chance of becoming a mature fruit than a bud on one of the larger inflorescences. The competition between individual buds on larger inflorescences seems to be too severe to allow all to survive. It is not, therefore, probable that the larger inflorescences are able to mature proportionally larger numbers of buds.

Heinicke [2] has recently reported that the reverse relationship exists in the case of the apple trees he studied. His figures indicated that a higher percentage of flowers develop into fruits on spurs producing six flowers each than on spurs producing four or five flowers. Further data on this question are to be desired.

[1] HARRIS, J. Arthur. THE CORRELATION BETWEEN A VARIABLE AND THE DEVIATION OF A DEPENDENT VARIABLE FROM ITS PROBABLE VALUE. *In* Biometrika, v. 6, pt. 4, p. 438–443. 1909.

—— CORRELATION IN THE INFLORESCENCE OF CELASTRUS SCANDENS. *In* Mo. Bot. Gard. 20th Ann. Rpt. p. 116–122. 1909.

[2] HEINICKE, Arthur J. FACTORS INFLUENCING THE ABSCISSION OF FLOWERS AND PARTIALLY DEVELOPED FRUITS OF THE APPLE (PYRUS MALUS L.). N. Y. Cornell Agr. Exp. Sta. Bul. 393, p. 41–114, illus. 1917. Bibliography, p. 112–114.

THE TIME REQUIRED FOR FRUIT TO DEVELOP FROM BLOSSOM TO MATURITY

Lemon fruits grow slowly in comparison with the fruits of most deciduous trees. About two months are usually required from the unfolding blossom until a lemon fruit reaches a diameter of ¼ inch. The time required for the fruit to reach a size of 2¼ inches (a desirable commercial size) varies according to conditions from 7 to 14 months. From the standpoint of the producer it is desirable to have the lemons reach mature size as soon as possible. A lemon which grows uniformly and rapidly is usually of superior quality. It reaches the size required for marketing without turning yellow to any appreciable extent; it develops the desired flavor after being artificially cured and withstands deteriorating influences during transportation and storage.

In the lemon the ovary begins to grow very soon after the perianth withers and falls off. The style adheres for some time after this, but eventually the stigma and a part of the style separate and fall away. "Time of maturity" is regarded as the time at which a lemon is harvested. This time is usually determined by one of two things, either the fruit has reached a diameter of 2¼ inches or it has lost all green color from its surface.

The records of 239 fruits were examined and the time at which they were set was ascertained. The observations on the time required for maturity are given in Tables VI and VII. It was found that the time required to mature individual lemons ranged from 7 to 14 months. The average time for all lemons in these records was 10.2 months. On account of the small number of lemons set in the months of November, December, January, and February, the figures for these months are omitted from Table VI, because of the inevitably larger error involved in averaging a few numbers. The reader will understand that fruit set in a given month was a bud in the preceding month. If this is borne in mind, there is no confusion in comparing Table VI with other tables in this paper.

TABLE VI.—*Average time required for growth of lemons to maturity according to the month in which fruit set*

Month in which fruit was set.	Number of fruits observed.	Mean time required for maturity. Number of months.	Month in which fruit was set.	Number of fruits. observed.	Mean time required for maturity. Number of months.
March	8	10.1±0.47	July	41	9.3±0.18
April	43	10.3± .16	August	23	11.7± .23
May	70	9.8± .10	September	10	11.6± .38
June	20	9.8± .30	October	9	11.7± .39

The table shows that there was a variation of about 2½ months in the average time required to produce a lemon, depending upon the month in which it set. Lemons which were set in May, June, and July came

to maturity in minimum time, and those set in August, September, and October required the maximum time.

Further light on the relation of the time of maturity to the time of setting was afforded by determining their correlation coefficient. Table VII shows the data. February is denoted as the first month in the subject column, since active growth begins in that month; and January is the last month.

TABLE VII.—*Correlation between the month in which lemons set and the time required for maturity*

Months required for maturity	7	8	9	10	11	12	13	14	Totals.
1	1	.....	.....	4	.....	.....	.....	.....	5
2	1	1	2	.....	.....	4	.....	.....	8
3	.....	11	1	5	19	6	.....	1	43
4	6	3	13	32	10	5	.....	1	70
5	1	3	8	2	3	3	.....	1	20
6	3	18	2	6	6	4	2	.....	41
7	1	.....	2	2	2	8	7	1	23
8	.....	.....	1	2	2	1	3	1	10
9	.....	.....	.....	.....	3	6	.....	.....	9
10	.....	.....	.....	.....	3	3	.....	.....	6
11	.....	.....	1	.....	.....	.....	.....	.....	1
12	.....	.....	1	.....	2	.....	.....	.....	3
	13	36	31	53	49	40	12	5	239

$r = 0.138 \pm 0.043$

The correlation coefficient denotes a positive relationship between the two factors, though its magnitude is not sufficient to warrant much emphasis. We can conclude that the season at which fruit is set influences, but does not absolutely determine, the length of time which will be required for maturity. Thus, fruit set in May had a range in time of maturing from 7 to 14 months, although about half the fruit required 10 months.

THE RATIO BETWEEN BUDS AND FRUIT WHICH REACHED MATURITY

The lemon may develop without pollination of the flower, therefore the proportion of fruit to buds may be expected to indicate the productiveness of the tree without entire dependence upon the chance of pollination as already intimated; however, the productiveness of the tree is greatly influenced by various environmental factors, especially by meteorological factors. Soil environment, as influenced by the application of fertilizers or water, affects fruit production; but its influence is not so abrupt and does not make itself quite so conspicuous as the former complex of factors.

An examination of these records may be of interest as an indication of what happens under good commercial culture. These trees stood in

a large plantation, receiving regular orchard treatment during the time observations were being made, and exposed to the vicissitudes which beset the commercial orchards. The effects of winter cold and of summer heat are plainly visible at places in the records, yet there is no reason to expect that the average lemon tree may escape the vicissitudes which befell these. The figures showing the proportion of buds which develop into mature fruit were based only upon observations made early enough to allow fruit to mature before the close of the observations.

Several definite stages in the development of fruit have been recognized in making this study. They are as follows: (1) the plump bud just ready to open; (2) the flower; (3) the first stage of the fruit at which the corolla has fallen but the style is still attached, designated for convenience "style attached"; (4) the young fruit having a diameter of ¼ inch and having lost the apical portion of the style; (5) the fruit having a diameter of 2¼ inches, ready to pick.

The individual histories of a random sample of lemon buds were followed from stage to stage to see what proportion survived and to locate, approximately, the time of heavy mortalities. A sample of 4,440 buds which appeared during the observation period was chosen. Their developmental history is shown in Table VIII.

TABLE VIII.—*Ratio of lemon buds to fruits which reached various stages*

Stage of development.	Number observed.	Per cent.
Buds ready to open	4,440	100.00
Styles attached	2,308	51.98
Fruit ¼ inch diameter	964	21.71
Fruit mature	294	6.62

These figures show that there is a large mortality between the young buds and the mature fruit, and that the mortality seems to increase with the age of the fruit. It should be stated, however, that losses were comparatively small after the fruit had reached a diameter of 1 inch. There is no reason, however, to regard the losses of fruit on these trees as abnormally high, since the crops produced on these trees were above the average for this district.

The effect of seasonal conditions upon the survival of young fruits seemed worthy of study in determining the ratio between buds and fruit. Since the time at which the fruit sets is the time at which it begins to grow, calculations were begun with the stage designated "style attached." Data were available for 2,453 fruits of this size which had the chance of developing into mature fruit during the time observations were made. Records were taken of the numbers of fruits reaching this stage in each month and of the numbers which matured from each of the several samples of "style attached" fruits.

TABLE IX.—*Relation of the survival of fruits to the months of the year in which they were set*

Month.	Number of "style-attached" fruits observed.	Mature fruits produced.	
		Number.	*Per cent.*
January	262	4	1.5
February	113	2	1.8
March	185	25	13.5
April	51	21	41.2
May	501	106	21.2
June	215	24	11.2
July	189	57	30.2
August	232	19	8.2
September	168	25	15.0
October	106	2	1.9
November	191	16	8.4
December	240	2	.8
Total	2,453	303	.....

It is apparent from these figures that there is considerable variability in the chances of survival, depending upon the time of year at which fruit is set. Fruit set in September or October is liable to be killed by cold weather in January, or if set in May or June is liable to be killed by hot weather in June. That which was set in the winter months was repeatedly chilled by low temperatures at night and its vitality was probably lowered. Fruit set in April and July appears to have the best chance of survival, though these relations may vary from one year to another.

It will perhaps be more nearly correct to group the records by seasons, since conditions are not necessarily confined to months.

TABLE X.—*Relation of survival to season at which fruit was set*

Season.	Number of "style-attached" fruits observed.	Mature fruits produced.	
		Number.	*Per cent.*
Spring: March, April, May	737	152	20.6
Summer: June, July, August	636	100	15.8
Autumn: September, October, November	465	43	9.2
Winter: December, January, February	615	8	1.3

These figures show that, upon the trees observed, a fruit set in one of the spring months had the best chance of survival and of reaching maturity. The chance of reaching maturity diminished as the seasons advanced until the next spring. On the other trees or in other localities the chances of survival might be quite different.

SUMMARY

(1) A small group of Lisbon lemon trees was studied for two years to obtain data upon their fruiting habits. The trees stood in a large commercial orchard and received no special treatment during the time observations were being made.

(2) Approximately 66 per cent of the fruit buds appeared during March and April, 13 per cent appeared in November, 17 per cent appeared between April and November, and about 3 per cent appeared during the winter months.

(3) The distribution of buds on an inflorescence showed no tendency to follow the normal curve of errors. Few-flowered inflorescences predominated numerically over many-flowered inflorescences. A bud on a small inflorescence had a greater chance of developing into a mature fruit than one on a large inflorescence. The competition between individual buds on larger inflorescences seems to be too severe to allow all to survive.

(4) The time required for the fruit to reach maturity varied from 7 to 14 months, according to conditions. Fruit which was set in May, June, and July came soonest to maturity. The season at which fruit was set appeared to influence, but not wholly to determine, the time which was required for maturity.

(5) The records for 4,440 buds showed that 51.98 per cent set fruit, 21.71 per cent reached a diameter of ¼ inch, and 6.62 per cent reached the stage of maturity.

(6) A fruit set in the spring months had the best chance of survival and of reaching maturity. The chances of reaching maturity diminished as the season advanced.

ULTRA-MICROSCOPIC EXAMINATION OF DISPERSE COLLOIDS PRESENT IN BITUMINOUS ROAD MATERIALS

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INTRODUCTION

On a cursory examination of bituminous solutions by means of the ultra-microscope, varying amounts of finely divided solid material held permanently in suspension will invariably be found. In certain hard native asphalts highly dispersed mineral matter is present in large quantities, and the high adhesive properties of such asphalts have been attributed largely to the selective absorption exerted by these colloids on certain portions of the bitumen.[1] Further investigations have led Richardson to conclude that some bitumens were absorbed in larger quantities, and consequently had a greater colloid-carrying capacity than others, and that this variation was apparently in accordance with their viscosity and the general character of the particular bitumen.[2]

As a result of these investigations, it seemed desirable to develop a reliable method of ultra-microscopic analysis whereby the number of disperse colloidal particles could be determined accurately in any type of bitumen, thereby furnishing a ready means for comparing their colloidal capacities and at the same time establishing a possible method for estimating the relative value of this property from a road-making standpoint. The fact, however, should be emphasized that the present investigations were undertaken essentially to develop a method for counting colloidal particles in bituminous solutions, and that in drawing comparisons of the relative supporting values from the results obtained, the original consistency of the materials employed should receive due consideration.

METHODS OF ULTRA-MICROSCOPIC EXAMINATION

When examined under the ordinary microscope, the great bulk of the colloidal material common to bituminous solutions is invisible. Early investigations by Siedentopf and Zsigmondy [3] have shown that the resolving power of the microscope is very greatly increased when particles are viewed in a powerful light against a dark background. This illumination was obtained originally by allowing a beam of light to enter the cell through a narrow slit at right angles to the axis of the instrument,

[1] RICHARDSON, Clifford. THE THEORY OF THE PERFECT SHEET ASPHALT SURFACE. *In* Jour. Indus. and Engin. Chem., v. 7, no. 6, p. 463-465. 1915.

[2] ———. IMPORTANCE OF THE RELATION OF SOLID SURFACES AND LIQUID FILMS IN SOME TYPES OF ENGINEERING CONSTRUCTION. *In* Sci. Amer. Sup., v. 83, no. 2152, p. 198-199. 1917. Printed also in separate form.

[3] ZSIGMONDY, Richard. ERKENNTNIS DER KOLLOIDE. 186 p. Jena, 1905.

where part of the rays were deflected from the surface of the suspended particles into the microscope, thus rendering them self-luminous and clearly visible while the remainder of the field remained dark. A similar effect may be obtained by means of a substage parabaloid condenser, with central stop, whereby the outer rays from the beam of light entering the microscope from below are brought by a series of reflections to a short focus within the cell and are totally reflected from the lower surface of the cell cover, leaving the field dark as before. Particles whose indices of refraction vary from those of the inclosing liquid intercepting these oblique rays diffract a portion of the light into the microscope and become luminously visible as in the former case while the remainder of the field is perfectly dark.[1]

In order to avoid loss of light through refraction of the rays issuing from the condenser, an immersion liquid, such as cedar oil or glycerin, should be employed between it and the cell containing the liquid under examination. This parabaloid illuminator is interchangeable with the substage Abbe condensor of the ordinary microscope and was consequently found most convenient for the present investigations. The microscope selected was provided with an accurately calibrated micrometer screw for vertical measurements and a mechanical stage for lateral orientation. A diamond point object marker with circular movement graduated to millimeters and insertible in the revolving nosepiece of the microscope will also prove a useful accessory. Light was furnished by a special arc lamp run on either direct or alternating current and regulated by a rheostat of 4.5 ampere capacity. Before entering the microscope the light was passed through a cooling solution, acting as a ray filter, of 10 mgm. diamine green dissolved in 1 liter of distilled water. A photograph of the microscope with arc light and ray filter used is shown in Plate 19, A.

The ordinary lens system of the microscope consisted of eyepieces ×7.5 and ×12.5 and objective 3, 4, and 16 mm., giving linear magnifications of 50 to 740 diameters at a tube length of 160 mm., while the best combination for counting was obtained with eyepiece ×7.5 and objective 4 mm., magnifying 320 diameters.

A counting device was inserted in the focal plane of this eyepiece, consisting of a cross-line micrometer scale with ground glass border divided into 25 square areas each side of which measured 1.25 mm. and corresponded exactly to 0.05 mm. of a stage micrometer at a tube length of 166 mm. With this micrometer the areal dimensions of any liquid under examination could be accurately determined, while the vertical element was obtained by means of the micrometer screw recording an interval of 0.00254 mm.[2]

[1] BURTON, E. F. THE PHYSICAL PROPERTIES OF COLLOIDAL SOLUTIONS. p. 46-47. London and New York. 1916.

[2] This micrometer was calibrated against that of a standard Fuess microscope registering a minimum vertical interval of 0.001 mm.

PREPARATION OF THE ULTRA-MICROSCOPE CELL

In order to carry out a quantitative analysis of bituminous solutions it was found necessary to employ a cell of minimum capacity that might be readily cleaned and hermetically sealed to prevent the escape of the volatile solvent. Efforts were made to utilize a container constructed on the principle of the Zeiss haemocytometer, as employed by Burton and Perrin in their examination of colloidal water solutions (hydrosols),[1] but it was found that all types of cement used in constructing this cell were attacked by the benzol solution and, furthermore, that the rulings on the bottom of the cell when filled with the solution were almost invisible under the microscope.[2]

In order to overcome the above-mentioned difficulties, it was found necessary to excavate a suitable cavity in the object glass itself, thereby doing away entirely with the superimposed glass plates of the haemocytometer slide. The object glass selected was as free as possible from air bubbles and other inclusions and had perfectly smooth plane surfaces and a thickness varying from 1.25 mm. to 1.75 mm. to assure a proper focus within the cell of rays from the dark field illuminator. he excavation was carried out by means of a stationary upright drill provided with a pointed vulcanized fiber cylinder having a flat grinding surface about 2 mm. in diameter. The drill was run by an electric motor at 1,800 revolutions per minute, using coarse emery mixed with a little heavy lubricating oil as an abrasive. In operating the drill great care was taken to apply a moderate uniform pressure, and the glass plate was protected from sudden strain by a folded towel or felt cushion placed beneath it. After grinding for one or two minutes the drill was removed and the cavity examined. In general, the central portion was found to be essentially flat and surrounded by deeper circular grooves, produced by the larger fragments of emery becoming lodged in the drill during the process of grinding.

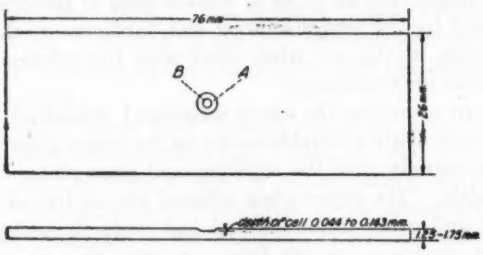
From this stage in the operation the grinding was carried on by means of an electrically driven, flexible shaft drill constructed on the principle of the dental drill and using volcanic ash or ground pumice with water as an abrasive. This drill, operating at a speed of 1,540 revolutions per minute, was provided with a grinding point of vulcanized rubber or belata gum which also proved very effective in polishing the cell. The polishing was begun with diatomaceous earth and water and

[1] BURTON, E. F. op. cit., p. 118–120.

PERRIN, Jean. MOUVEMENT BROWNIEN ET RÉALITÉ MOLÉCULAIRE. *In* Ann. Chim. et Phys., s. 8, t. 18, p. 40–42. 1909.

[2] The indices of refraction for ordinary light flint glass and benzol at 21.5° C. are 1.5710 (D) and 1.5304 (H), respectively. SMITHSONIAN PHYSICAL TABLES, ed. 6, p. 184, 192. Washington, D. C., 1914. (Smithsn. Misc. Collect., v. 63, no. 6.) Hence light passing through glass and meeting etched lines on a cell bottom mounted in benzol are but slightly diffracted and consequently appear indistinct under ultra-microscopic illumination while plainly visible when viewed in water or air.

was continued with a mixture of freshly precipitated calcium carbonate and magnesia hydrate until a microscopically smooth and essentially flat surface was obtained. Finally, in the center of the cell a circle 1 mm. in diameter was inscribed with the diamond-point marker of the microscope in order to limit the field of observation. A diagram of the slide, with 2½ mm. cell (A) containing circular area (B) drawn to natural scale, is shown in figure 1, where the depth is indicated as lying between 0.044 and 0.143 mm. These values were determined as accurately as



A is saucer-shaped cell 2½ mm. in diameter.
B is circular field 1 mm. in diameter in center of cell floor.

FIG. 1.—Glass slide with ultra-microscope cell drawn to natural scale.

possible for each cell by means of a strain dial recording intervals of 0.0001 inch and were checked with the microscope micrometer under a magnification of 740 diameters. A blunted, highly polished needle point inserted in the vertical arm of the dial enabled readings to be taken at different points within the cell, and the average of these readings was compared with that of an equal number taken around the cell from without. The results of these measurements for a number of cells constructed in the manner outlined above, together with microscopic check determinations, inclosed in parentheses, are given in the following table:

TABLE I.—*Depth of ultra-microscope cells determined by strain dial*

Slide number.	Depth in mm.	Thickness of slide in mm.	Maximum variation in depth in mm.	Maximum variation in thickness in mm.	Percentage of variation in depth.	Percentage of variation in thickness of slide.
1	0.1209 [a] (.1220)	1.5316	0.0058	0.0061	4.83	0.40
2	.1321	1.5382	.0160	.0101	12.10	.66
3	.0434 (.0423)	1.7374	.0036	.0096	8.30	.55
4	.1432 (.1450)	1.6103	.0094	.0046	6.50	.28
5	.0795 (.0790)	1.4880	.0071	.0053	9.20	.36
6	.0892 (.0890)	1.6812	.0043	.0061	4.82	.36
Average	.1014	1.5978	.0077	.0070	7.53	.43

[a] Determined by microscope micrometer.

In comparing these values it will be noted that the average depth of all cells is but slightly in excess of 0.10 mm., while the maximum variation in depth and in the thickness of slide is approximately the same (0.007 mm.), indicating a cell floor closely approaching a true plane.

Dial measurements also were undertaken to determine the depth of cell, including cover glass after mounting in the asphaltic oil solution employed in counting (see below) and in air to form an estimate of the relative thickness of the liquid film between cover glass and slide beyond the cell area. In every case lesser values were obtained for cells mounted in this solution than in air, indicating a more perfect contact through the release of atmospheric pressure and the adhesive character of the bitumen.

PREPARATION OF SOLUTIONS

Before describing the method of counting colloidal particles employed in this investigation, it will be found desirable to outline briefly the general character of bituminous solutions containing colloidal matter and the manner in which these solutions have been prepared for microscopic analysis.

When viewed under the ultra-microscope the colloidal portion of the solution will appear as a mass of very finely divided and more or less widely dispersed particles undergoing a constant and, under certain conditions, perpetual movement (Brownian movement). This movement has been ascribed to the molecular energy of the suspending liquid and may be regarded as a function of the size of the particles and their degree of dispersion which, in turn, is limited by the viscosity of the solution.[1] In order, therefore, to count successfully these suspended particles it was found necessary either to retard their movement by suitable concentration of solution or to increase it by dilution to such a degree that they settled out within the cell inclosure.[2] In the latter case, however, it frequently happened that the particles were in part resorbed on exposure to light, thus destroying the accuracy of the count. To assure concordant results, therefore, the particles were always counted in a somewhat viscous solution of colloid-free asphaltic oil to which a definite amount of paraffin had been added. This solution was prepared by fluxing 2.5 gm. Mexican oil asphalt (penetration 148) with 0.5 gm. crystalline paraffin and diluting to 100 cc. with benzol containing 10 per cent alcohol. This was then evaporated to constant weight on the water bath, brought to original consistency with benzol and passed through an alundum tube or filter tube clogged with macerated filter paper until approximately all suspended matter had been removed.

[1] OSTWALD, Wolfgang. DIE WELT DER VERNACHLÄSSIGTEN DIMENSIONEN. p. 34–35. Dresden and Leipzig, 1915.

[2] The solvent used in these investigations was c. p. benzol, since carbon bisulphid was found to contain an appreciable quantity of colloidal sulphur.

When properly prepared, the diluting solution should contain not more than 10 particles to a ¼ mm. square field in cell No. 3, at a magnification of 320 diameters. A definite portion of the solution to be examined was introduced into this standard dilutant after the coarser mineral matter had been removed. That was accomplished by dissolving 1 gm. of the original sample in 50 cc. benzol in a stoppered centrifuge tube, allowing the solution to stand overnight (17 hours) and centrifuging for 1 hour at a speed of 800 revolutions per minute. A small portion of this solution was then drawn off from the tube at a depth of 10 mm., and 1 cmm. transferred to a glass-stoppered graduate and brought up to 10 cc. with the paraffin oil dilutant. By this means a dilution of 1 to 5,000 of the colloids present in the original sample was obtained. This was found to be sufficient in most cases, but in certain bitumens where the dispersed mineral matter was in a state of extreme subdivision a further dilution of 1 to 50,000 was necessary before the colloid particles could be conveniently counted.

METHOD OF COUNTING COLLOIDAL PARTICLES

The samples of bitumens selected for examination were obtained from the commoner types of road material, ranging from hard native asphalt to lighter oils and containing varying amounts of colloidal matter. After having been subjected to the preliminary treatment mentioned above, one or two drops of the properly diluted solution were rapidly transferred from the 10-cc. graduate to the cell by means of a 1-cc. pipette and covered immediately by a 18-mm. cover glass, using a bluntly pointed wooden rod to expel all excess liquid and assure a perfect contact between slide and cover glass. After the excess solution had hardened sufficiently by evaporation and the slide beyond the cell limit appeared perfectly clear and colorless, the cover glass was sealed with a 30 per cent solution of boiled Canada balsam in ether applied with a hair-line paintbrush (No. 0). A photomicrograph of a part of the mounted cell with cross-line micrometer scale magnified 320 diameters is shown in Plate 19, B.

In order to obtain consistent results, the cell and cover glass should be microscopically clean before mounting and the dilutant examined from time to time to allow for corrections in the final results.[1] When properly mounted the cell should be free from air bubbles and the colloidal particles should appear under the microscope evenly distributed and in constant, though restricted, motion. In correct focus these particles were clearly defined as brilliant points of light against a dark background, but a change of focus resulted in the development

[1] The cleaning was accomplished by first boiling slide and cover glass in concentrated sulphuric acid, then rinsing in water, alcohol, and benzol, drying with soft cotton or silk cloth, and rubbing with optical tissue paper until thoroughly clean.

of concentric halos or diffraction rims around each particle that detracted greatly from the definition of the images. In order to overcome this so far as possible, it was found necessary to employ cells below 0.10 mm. in depth, having a capacity less than 0.10 cmm. (Table I, No. 3, 5, and 6). Counts were made of all particles in suspension as well as those that might have settled out on the cell floor or become attached to the cover glass during the process of counting. The area examined was taken from within the central millimeter circle of the cell and represented exactly one-fourth of 1 square millimeter (equivalent to four fields of the cross-line eyepiece micrometer at a magnification of 320 diameters), while the volume of liquid was obtained from this area and the depth of cell employed (0.043–0.089 mm.). The number of particles counted in each of the 25 square subdivisions of the micrometer through the entire depth of liquid was recorded, and from the average of four such determinations the value for 1 cmm. of solution and 1 gm. of bitumen was computed.

The results of the analyses were recorded on a special form which, in addition to the data indicated above, contained information regarding the physical properties of the bitumen, together with the relative size and distribution of the colloidal particles. In general, it may be stated that these particles varied in size from submicrons having a minimum diameter of about 15 $\mu\mu$ (0.000015 mm.) to particles within the visibility of the ordinary microscope (above 0.25 μ=0.00025 mm.).[1] These dimensions may be determined by direct microscopic measurement or they may be calculated by dividing the total volume of particles contained in a definite quantity of solution by the number of particles found where the volume represents the weight of the particles divided by their specific gravity. The quotient thus obtained will equal the volume of one particle (x). Assuming the particles to be spheres of diameter a,

$$\text{Then } \frac{4}{3}\pi a^3 = x$$

$$a = \sqrt[3]{\frac{x}{\frac{4}{3}\pi}}$$

RESULTS OF THE ULTRA-MICROSCOPIC EXAMINATION OF BITUMINOUS SOLUTIONS

In order to standardize the method of ultra-microscopic analysis outlined above, a number of determinations were made of various colloidal materials contained in different types of bitumens. The results of these determinations are shown in Table II.

[1] ZSIGMONDY, Richard. OP. CIT., p. 88b–97.

TABLE II.—*Ultra-microscopic counts of colloidal particles in bituminous solutions*

Sample No.	Cell No.	Depth of cell in mm.	Bitumens used.	Consistency before treatment.		Consistency after treatment.	Number of particles counted per cmm. at 1:5000 dilution.	Average per cmm.	Maximum variation.	Per centage of variation.	Supporting value.
				Penetration.	Specific viscosity.	Penetration.					
1	3	0.0444	Refined Trinidad asphalt	2			400,000				
2	3	.0444	....do	2			405,584				
3	5	.0802	....do	2			416,000	406,256	16,000	3.94	100.8
4	6	.0892	....do	2			400,726				
5	6	.0892	....do	2			408,968				
6	3	.0444	Refined Trinidad asphalt and clay				30,000				
7	3	.0444	....do				32,000				
8	5	.0802	....do				29,120	30,529	2,880	9.43	7.5
9	6	.0892	....do				31,031				
10	6	.0892	....do				30,495				
11	3	.0444	Trinidad petroleum residuum and clay		105 at 25° C	235	167,630				
12	5	.0802	....do		....do	235	169,260				
13	5	.0802	....do		....do	235	163,670	169,262	16,082	9.50	41.6
14	6	.0892	....do		....do	235	179,752				
15	1	.1370	....do		....do	235	166,000				
16	3	.0444	Mexican oil asphalt and clay	145		13	106,000				
17	5	.0802	....do	145		13	106,786	108,113	4,020	3.21	26.6
18	6	.0892	....do	145		13	109,417				
19	6	.0892	....do	145		13	110,135				

TABLE II.—*Ultra-microscopic counts of colloidal particles in bituminous solutions*—Continued.

Sample No.	Cell No.	Depth of cell in mm.	Bitumens used.	Consistency before treatment.		Consistency after treatment.	Number of particles counted per cmm. at 1:5000 dilution.	Average per cmm.	Maximum variation.	Percentage of variation.	Supporting value.
				Penetration.	Specific viscosity.	Penetration.					
20	3	.0444	Mexican oil asphalt, 16 per cent $Cu.CO_3.Cu(OH)_2$	57		8	2,892,000	2,858,000	92,000	3.29	703.5
21	3	.0444	do	57		8	2,800,000				
22	3	.0444	do	57		8	2,892,000				
23	3	.0444	Trinidad petroleum residuum, 16⅔ per cent $CuCO_3.Cu(OH)_2$.		220 at 100° C.	18	1,089,900	1,085,950	7,900	.73	267.3
24	5	.0802	do		do	18	1,082,000				
25	3	.0444	Mexican oil asphalt, 10 per cent $CuCO_3.Cu(OH)_2$	57		10	1,033,200	1,012,800	108,000	11.25	249.3
26	5	.0802	do	57		10	990,000				
27	3	.0444	do	57		10	960,000				
28	5	.0802	do	57		10	1,068,000				
29	3	.0444	Trinidad petroleum residuum, 10 per cent $CuCO_3.Cu(OH)_2$		226 at 100° C.	25	805,000	785,500	39,000	5.09	193.3
30	5	.0802	do		do	25	766,000				
31	3	.0444	Mexican oil asphalt, 20 per cent $Pb(C_2H_3O_2)_2.3H_2O$	250		70	284,800	262,400	44,800	18.7	64.6
32	5	.0802	do	250		70	240,000				
33	3	.0444	Mexican oil asphalt, 10 per cent $CuSO_4$	57		16	209,000	204,160	18,000	9.2	50.2
34	6	.0892	do	57		16	214,000				
35	6	.0892	do	57		16	201,800				
36	3	.0444	do	57		16	200,000				
37	5	.0802	do	57		16	196,000				
38	3	.0444	Gilsenite, 10 per cent $CuCO_3.Cu(OH)_2$				101,442	100,721	1,442	1.44	24.8
39	3	.0444	do				100,000				
40	3	.0444	Water gas tar, 20 per cent $CuCO_3.Cu(OH)$		(a)	21	20,000	20,455	2,536	12.4	5.0
41	3	.0444	do			21	19,420				
42	5	.0802	do			21	21,956				
43	3	.0444	Mexican oil asphalt, 10 per cent $Fe.SO_4$	57		28	20,000	18,667	2,000	10.7	4.6
44	3	.0444	do	57		28	18,000				
45	5	.0802	do	57		28	18,000				
46	3	.0444	Mexican oil asphalt, 10 per cent $ZuSO_4$	57		16	4,248	4,459	422	9.46	1.1
47	5	.0801	do	57		16	4,670				

[a] Float test=153″ at 32° C.

It will be noted that duplicate check counts were made generally in cells of varying capacity, giving the maximum numerical and percentage variation and indicating as well the supporting value or colloidal capacity of each type of bitumen, based on that of refined Trinidad asphalt considered as 100. All results were computed on a basis of 1 to 5,000 dilution of the original colloidal portion of the sample. (See p. 175.) These bitmuens have been separated into groups containing clay as colloidal material (No. 1–19) and into others in which this mineral matter was replaced by carbonates, sulphates and acetates of copper, iron, zinc, and lead (No. 20–47). The samples included in the first class, except untreated refined Trinidad asphalt (No. 1–5), were prepared by incorporating 33 per cent sandy clay in each type of bitumen by Richardson's method of heating an aqueous emulsion of clay and bitumen until all moisture and gas had been expelled.[1] In the case of refined Trinidad asphalt and clay (No. 6–10) all insoluble mineral and organic matter originally present in the bitumen was removed before emulsifying by dissolving in benzol and adding about 2 per cent shellac dissolved in alcohol and evaporating to constant weight, redissolving in benzol and filtering through an alundum tube until the solution was essentially void of colloidal particles.

In the second group of bitumens (No. 20–47) the salts were introduced in an anhydrous condition and the mixtures were heated to about 170° C. under constant stirring until all evolution of gas had ceased. On examining solutions of this kind under the ultra-microscope it was found that the copper carbonate salts had been largely reduced to red cuprous oxid, accompanied by an enormous colloidal dispersion (No. 20–30), while with the remaining salts the reduction had been much less complete (No. 31–39) or entirely lacking (No. 40–47), and the development of colloids correspondingly less. It may be stated, therefore, that the colloidal capacity of the second group of materials taken as a whole was dependent largely upon the degree of chemical reaction between the bitumen and the salts employed, while in the first group this supporting value was related more directly to the physical character of the bitumen.

A comparison of the duplicate counts recorded in columns 3 and 4 of the table indicated that a maximum variation of less than 10 per cent was attained in samples of the first group (No. 1–19), while in the second group (No. 20–47) the results were, on the whole, less concordant, owing largely to the greater dispersion of colloidal matter.

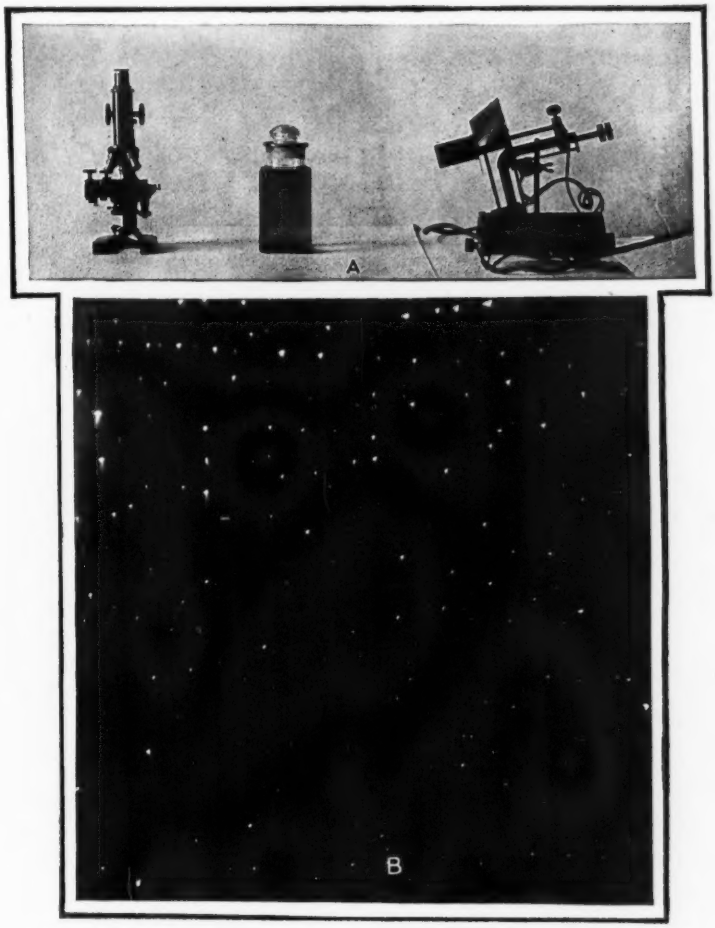
In conclusion, it may be stated that the accuracy of this method for counting colloidal particles in bituminous solutions depends chiefly upon accuracy in construction and calibration of the cell employed, as well as upon the proper consistency and optical purity of the supporting liquid.

[1] RICHARDSON, Clifford. 1917. OP. CIT.

PLATE 19

A.—Microscope with ray filter and arc lamp for dark field illumination.

B.—Photomicrograph of cross-line micrometer scale, showing colloids in dark field. ×320. Taken by E. A. Shuster, jr., Photographic Laboratory, United States Geological Survey.


A
B